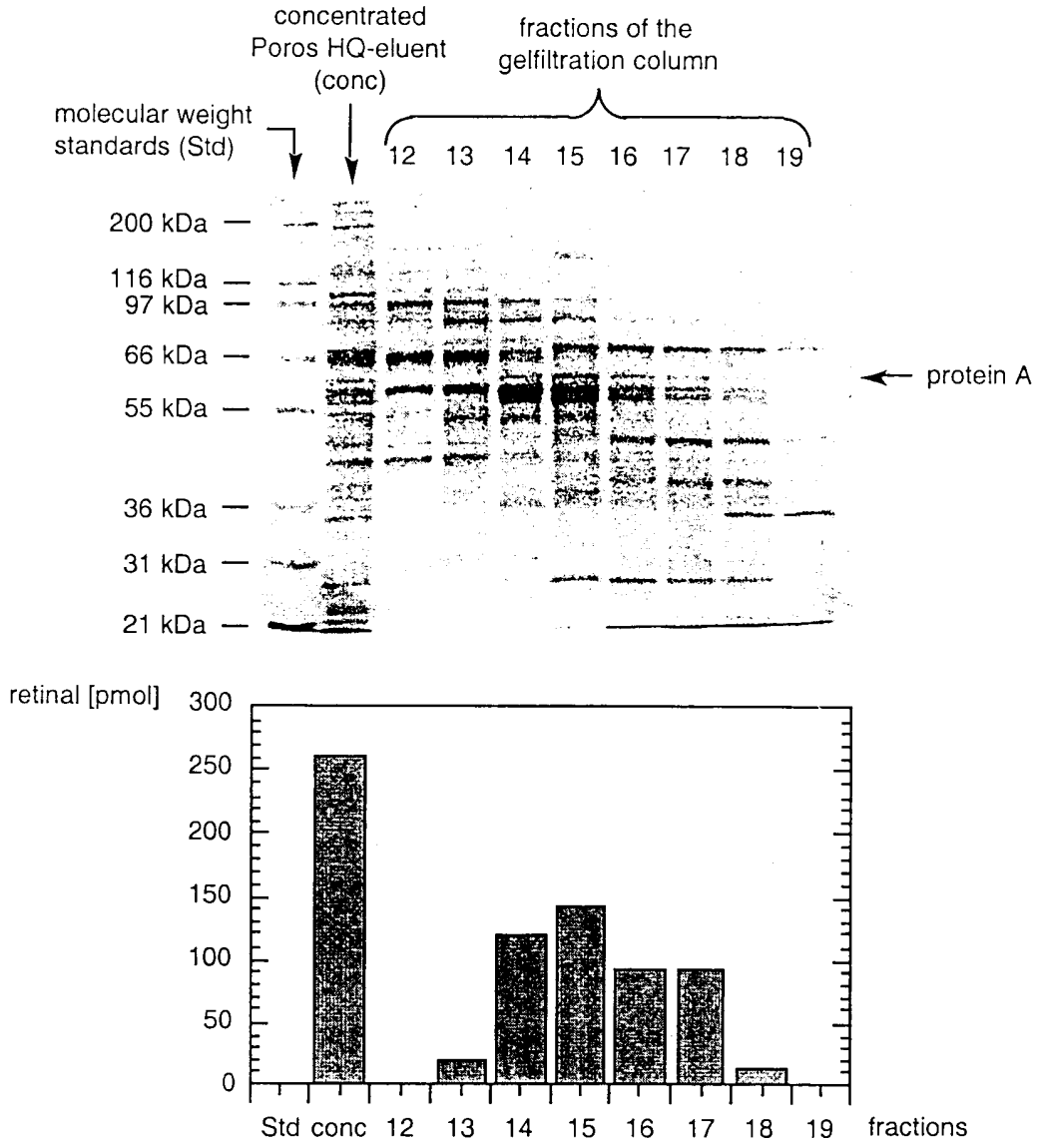
Figure 1


concentrated
Poros HQ-eluent
(conc)
fractions of the
gelfiltration column
molecular weight
standards (Std)
12 13 14 15 16 17 18 19
200 kDa
116 kDa
97 kDa
66 kDa
55 kDa
36 kDa
31 kDa
21 kDa
protein A
retinal [pmol]
300
250
200
150
100
50
0
Std conc 12 13 14 15 16 17 18 19
fractions

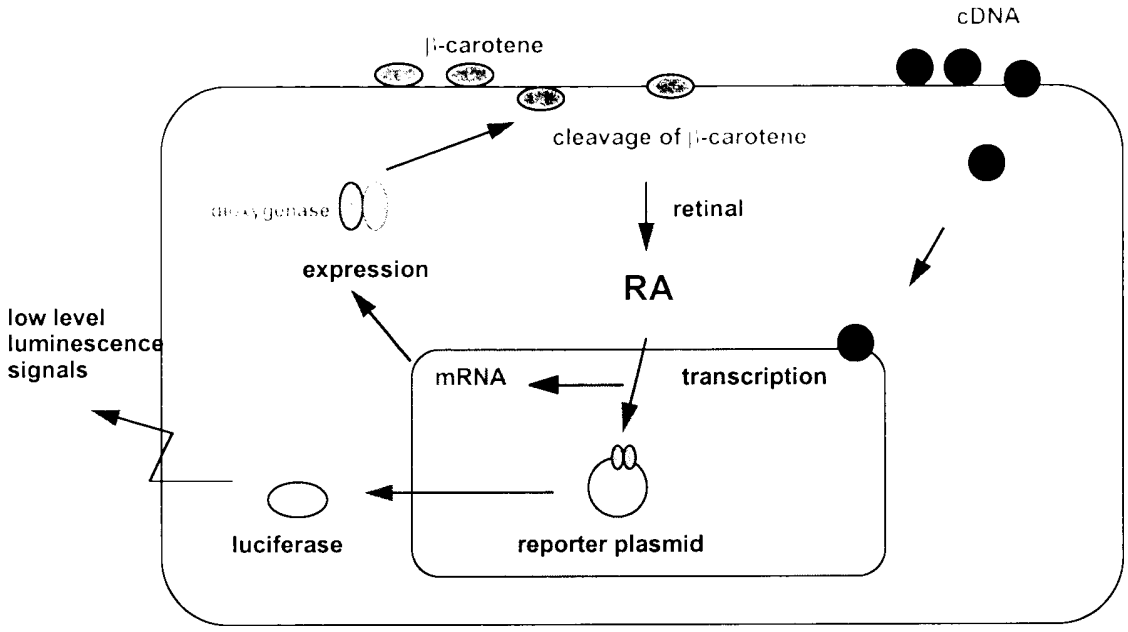

cDNA
β-carotene
cleavage of β-carotene
retinal
RA
expression
low level
luminescence
signals
mRNA
transcription
luciferase
reporter plasmid

Figure 2

Figure 3 Seq. ID No. 2

```
       1  CGGATCCACT AGTAACGGCC GCCAGTGTGG TGGAATCCAT
CCTTCTATGT

      51  AACAGGAAAG AGCTGTTCTT AGCCCAGAGA GGAGGGCACC
GTACGCCTGC

     101  AGGAGCAGCT GGGTAGAGGA CACAGGAGAG CGATGGAGAC
AATATTTAAC

     151  AGAAACAAAG AAGAGCATCC AGAGCCCATA AAAGCTGAGG
TGCAAGGTCA

     201  GTTGCCCACT TGGTTGCAAG GGGTACTTCT CCGAAATGGC
CCAGGGATGC

     251  ACACAATAGG GGACACTAAA TACAACCACT GGTTTGATGG
CTTGGCTCTG

     301  CTGCACAGCT TCACGTTTAA AAATGGTGAA GTTTACTACA
GAAGTAAGTA

     351  CCTCCGAAGT GACACATACA ACTGCAATAT AGAAGCAAAC
CGAATCGTGG

     401  TGTCTGAGTT TGGAACCATG GCTTATCCGG ATCCATGCAA
AAACATATTT

     451  GCCAAGGCAT TCTCATACTT ATCTCACACC ATTCCTGAGT
TCACGGACAA

     501  CTGCCTGATC AACATTATGA AAACTGGGGA TGATTATTAT
GCTACCAGTG

     551  AGACTAACTT CATCAGAAAA ATTGATCCAC AGACTCTGGA
GACACTAGAT

     601  AAGGTAGACT ACAGCAAATA TGTAGCTGTA AACTTGGCAA
CTTCTCACCC

     651  ACACTATGAC AGTGCTGGAA ATATTCTCAA CATGGGTACT
TCAATTGTTG

     701  ATAAAGGGAG AACAAAATAT GTTCTCTTTA AGATCCCTTC
CTCTGTACCA

     751  GAAAAAGAAA AGAAGAAATC TTGTTTTAAA CACCTGGAAG
TAGTATGCTC

     801  CATCCCTTCT CGCTCCCTGC TCCAACCAAG CTACTACCAC
AGCTTTGGAA

     851  TCACAGAAAA TTATATTGTG TTCATAGAGC AGCCATTTAA
ACTGGATATT
```

901 GTCAAACTGG CAACTGCCTA CATCCGAGGT GTGAACTGGG
CTTCCTGCCT

951 TTCCTTTCAT AAGGAGGATA AGACGTGGTT TCACTTTGTA
GACAGAAAGA

1001 CGAAAAAAGA AGTATCCACC AAGTTTTACA CTGATGCTTT
GGTGCTTTAT

1051 CACCACATAA ATGCTTACGA AGAAGATGGC CACGTTGTTT
TTGATATCGT

1101 TGCCTACAGA GACAATAGCT TGTACGATAT GTTTTACTTA
AAAAAACTGG

1151 ACAAAGACTT TGAAGTGAAC AACAAGCTTA CCTCCATCCC
AACCTGCAAG

1201 CGCTTTGTTG TGCCTCTGCA GTATGACAAG GATGCAGAAG
TAGGTTCTAA

1251 TTTAGTCAAA CTTCCAACTT CCGCAACTGC TGTAAAAGAA
AAAGATGGCA

1301 GCATCTATTG TCAACCTGAA ATATTATGTG AAGGGATAGA
ACTGCCTCGT

1351 GTCAACTATG ACTACAATGG CAAAAAATAC AAGTATGTCT
ATGCAACAGA

1401 AGTCCAGTGG AGCCCAGTTC CTACAAAGAT TGCAAAACTG
AATGTCCAAA

1451 CAAAGGAAGT ACTGCACTGG GGAGAAGACC ACTGCTGGCC
CTCAGAGCCC

1501 ATCTTTGTTC CCAGCCCCGA TGCAAGAGAA GAGGATGAAG
GTGTTGTTTT

1551 GACCTGTGTT GTGGTGTCTG AGCCAAATAA AGCACCCTTC
CTACTCATCT

1601 TGGATGCTAA AACATTCAAA GAATTGGGCC GAGCCACAGT
TAACGTAGAA

1651 ATGCATCTGG ACCTGCATGG GATGTTTATA CCACAGAATG
ATTTGGGGGC

1701 TGAGACGGAA TAAAACGCTA TTGATCCGAC TACACAAACT
GAGACAACTT

1751 TCTACTGAAC ATGAGTTAAT ATCCCTTTTA CCATTCAAGA
ACAACCATAT

1801 AACGACACAA AATGACTATG TATAATCTCT TAAATAATAG
ATATAATCCT

1851 TTTAAGGCAC AGCGATGAGT TTTACTACAG GTAACGATAT
GCACAACTGG

1901 CATATAACTA TTCCAAAAGA AGAAGAACGA TCAGTGTTTT
AGAAGTGCTA

1951 ATGTTGTACA TAACGGCGGC AGAGGGAACA GGAGAGAAAG
GTAACGGGAA

2001 TATTTAATAG AATATAGATT TCTGAGCAAA TGAAGTGCAG
TATTTATGGT

2051 GTGATGCATG GCATGAGTCA CATAGGTCTG CAGCTCATGT
ATCTTTTAGA

2101 GATCGTTTCA AGATTGCAGC TTGTGATGCA AGTTTTCTCC
AGCCAGAAAA

2151 CCTCATTTTA AACCATCTGC TACTGGTAAT TCATACCAAT
GCATTTTCTT

2201 GGTGCTCGAT TTACACTATA ACCAAAGTTA AGTATTACAT
TCAGGTGCTA

2251 CAACTTTCTA ATTTACAACC GAAACAAACA AGCAAACAGC
ACTTGCTTTG

2301 CTAATAACCC CATGGTGTAT TTTTCCTTTT TATGATGACA
AAACCAAGTA

2351 CATATGGTTT TATGTAGCAT TCAATTATAC TTCAGTGCTA
TTCCATCCTA

2401 ATGTTATAAG CAATTTGTAT TTAAATCAGT TTTCCTTGAG
AATATCTGAC

2451 ATAACATTTT GTGTAATGAG ATGACTATGT TGTCTAAAGA
TGAACAGGAA

2501 TGTATCTTTT ATTAGTATTG TTAATTGTGT TACTAATACT
ATGCATATGA

2551 ATGAGAGCAA TGTATTTCTA GGAGAACTCA GATATACATT
CAACAATTTC

2601 TGTAGGTGAA AATGCATTTA CTGATGAAAG TTGAATCGTT
AATGAGGGAG

2651 AAAACTGGGT ATCCATCCAT CCAACTATGT TAGGTGTTCA
CCTGGTCTGT

2701 ATGTGACACC ACGCTGTTTG GGTATCTCTC ACTTTCACAT
ACCTGTTCTC

2751 ATGGTTTCTG CTACTCACTG TATTTTGCAG GAGAGAAACA
AAATGAAATC

2801 ACTGTCACTT ACTATCGCCC CATCACATAA GAACAATGGG
GCTTTGGTGA

2851 CTTGTTCATG ATTACATAAG ATGTTTGCAG CAGAGCAGCA
ATAGAACCAA

2901 CACCATCCAC AGTTCTTGCT TGCTCTGTTA TGACTCCCTT
TGCTGTCTTT

2951 ATGGTTTGCA TGTATGAAGA ATACACTGCC TAATTCTAAT
GTTAAAAAGT

3001 CACTGGGGTC AGATCTAGAG CTTAAGTAAG CAGTCTGGGG
TTTTCAAATG

3051 TTTATATGTT CCATAAAATG GAAATAAACA CCTCCATAAT
AAAAAAAAAA

3101 AAAAAAAAAA A

Figure 4 Seq. ID No. 1

1 METIFNRNKE EHPEPIKAEV QGQLPTWLQG VLLRNGPGMH TIGDTKYNHW

51 FDGLALLHSF TFKNGEVYYR SKYLRSDTYN CNIEANRIVV SEFGTMAYPD

101 PCKNIFAKAF SYLSHTIPEF TDNCLINIMK TGDDYYATSE TNFIRKIDPQ

151 TLETLDKVDY SKYVAVNLAT SHPHYDSAGN ILNMGTSIVD KGRTKYVLFK

201 IPSSVPEKEK KKSCFKHLEV VCSIPSRSLL QPSYYHSFGI TENYIVFIEQ

251 PFKLDIVKLA TAYIRGVNWA SCLSFHKEDK TWFHFVDRKT KKEVSTKFYT

301 DALVLYHHIN AYEEDGHVVF DIVAYRDNSL YDMFYLKKLD KDFEVNNKLT

351 SIPTCKRFVV PLQYDKDAEV GSNLVKLPTS ATAVKEKDGS IYCQPEILCE

401 GIELPRVNYD YNGKKYKYVY ATEVQWSPVP TKIAKLNVQT KEVLHWGEDH

451 CWPSEPIFVP SPDAREEDEG VVLTCVVVSE PNKAPFLLIL DAKTFKELGR

501 ATVNVEMHLD LHGMFIPQND LGAETE

Figure 5 Seq ID No. 4 and Seq ID No. 5

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      10 EEHPEPIKAEVQGQLPTWLQGVLLR..NGPGMHTIGDTKYNHWFDGLALL
57
      20 EELSSPLTAHVTGRIPLWLTGSLLRCFTGPGLFEVGSEPFYHLFDGQALL
69
      58 HSFTFKNGEVYYRSKYLRSDTYNCNIEANRIVVSEFG..TMAYPDPCKNI
105
      70 HKFDFKEGHVTYHRRFIRTDAYVRAMTEKRIVITEFGFTTCAFPDPCKNI
119
     106 FAKAFSYLSHTIPEFTDNCLINIMKTGDDYYATSETNFIRKIDPQTLETL
155
     120 FSRFFSYFRGV..EVTDNALVNVYPVGEDYYACTETNFITKINPETLETI
167
     156 ..DKVDYSKYVAVNLATSHPHYDSAGNILNMGTSIVDKGRTKYVLFKIPS
203
     168 FTKQVDLCNYVSVNGATAHPHIENDGTVYNIGNCFGKNFSIAYNIVKIPP
217
     204 SVPEKEKKKSCFKHLEVVCSIPSRSLLQPSYYHSFGITENYIVFIEQPFK
253
     218 LQADKEDPISKFTS.EIVVQFPCSDRFKPSYVHSFGLTPNYIVFVETPVK
266
     254 LDIVKLATAY.IRGVNWASCL.SFHKEDK.TWFHFVDRKTKKEVSTKFYT
300
     267 INLFKFLSSWSLWGANYMDCFESFTNETMGVWLHIADKKRKKYLNNKYRT
316
     301 DALVLYHHINAYEEDGHVVFDIVAYRDNSL...YDMFYLKKLDKDFE...
344
     317 SPFNLFHHINTYEDNGFLIVDLCCWKGFEFVYNYFTLYLANLRENWEEVK
366
     345 VNNKLTSIPTCKRFVVPLQYDKDAEVGSNLVKLP.TSATAV..KEKDGSI
391
     367 KNARKAPQPEVRRYVLPLNIDK.ADTGKNLVTLPNTTATAILCSDEFTTI
415
     392 YCQPEILCEG....IELPRVNYD.YNGKKYKYVYATEVQWSPVPTKIAKL
436
     416 WLEPEVLFSGPRQAFEFPQINYQKYCGKPYTYAYGLGLNHF.VPDRLCKL
464
```

```
     437 NVQTKEVLH..WGEDHCWPSEPIFVPSPDAREEDEGVVLTCVVVSEPNKA
484
         ||.|||     | |   :|||||||  ||| |||:||||. ||     .
     465 NVKTKETWFTVWQEPDSYPSEPIFVSHPDALEEDDGVVLSVVVSPGAGQK
514
                    .          .          .
     485 P.FLLILDAKTFKELGRA..TVNVEMHLDLHGMF 515
         | :||||.||   |. ||  || : . .  ||:|
     515 PAYLLILNAKDLSEVARAEFTVEINIPVTFHGLF 548
```

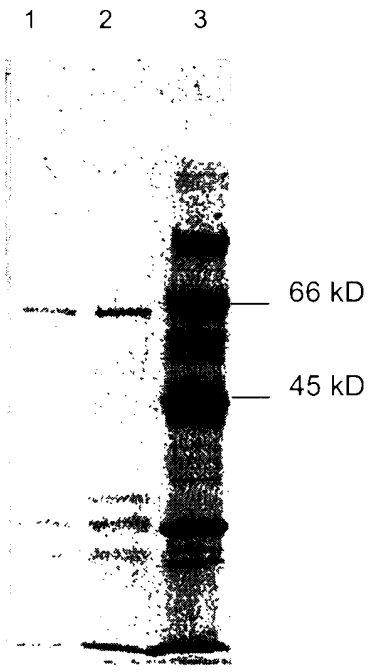

Fig. 6 shows a 10% polyacrylamide gel with E.coli expressed β,β-carotene 15,15'-monooxygenase after affinity tag purification; lane 1 and lane 2: 2 fractions from the Co^{2+}-chelate column showing the main band at 60 kD; lane 3: low range molecular weight marker (Bio Rad).

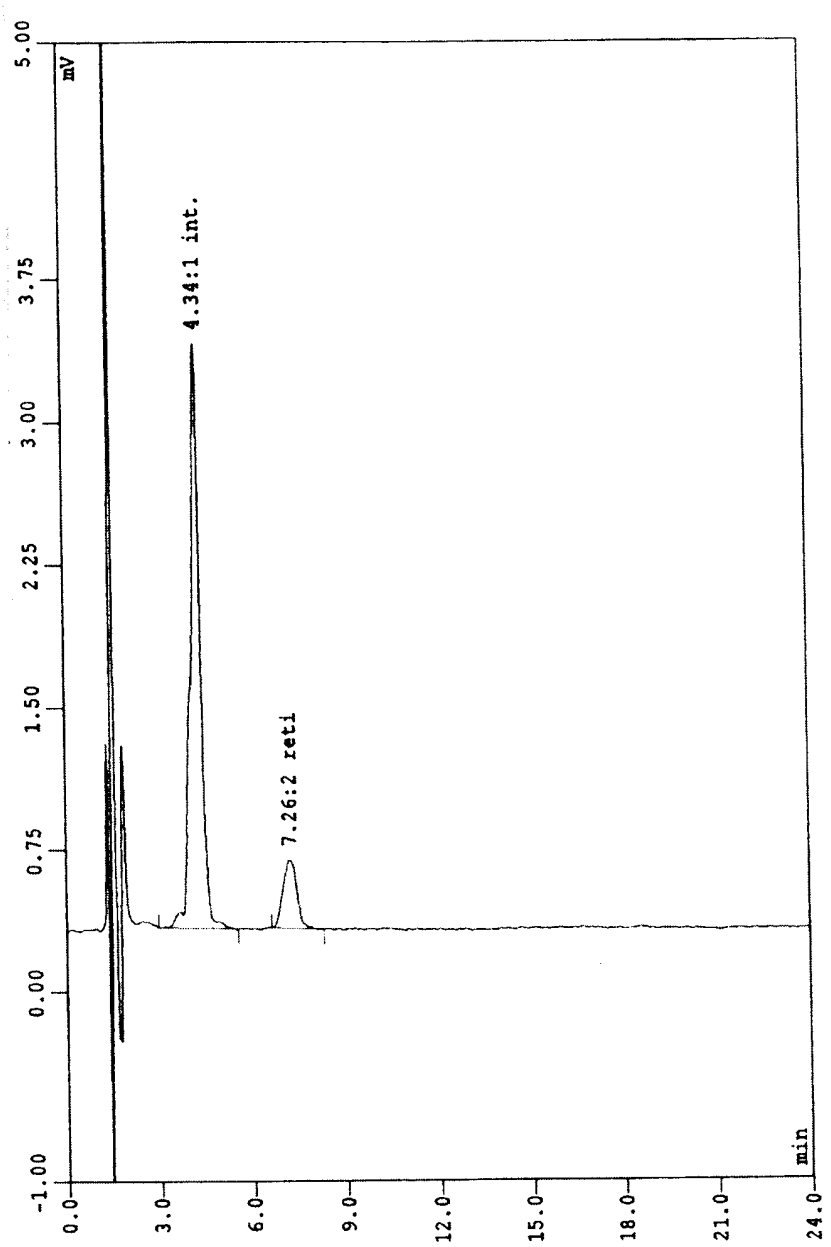

Fig. 7 shows an HPLC profile of the reaction mixture at the end of an actvity assay for the β,β-carotene 15,15'-monooxygenase following the procedure in example 1. The first peak in the chromatogram represents the internal standard, while the second peak corresponds to retinal as the only product formed during the central cleavage with β-carotene as substrate.

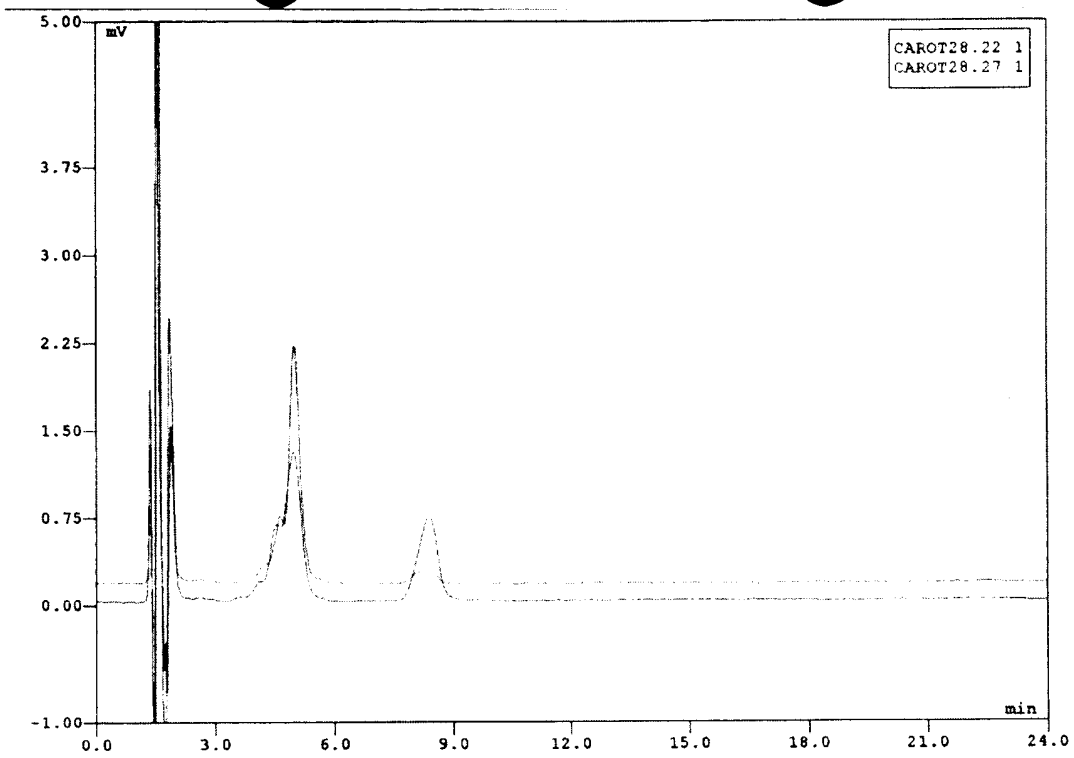

Fig. 8 confirms that the product peak in Fig. 7 is indeed retinal. A sample which was positive in the activity assay (green (upper) chromatogram) was spiked with retinal and analysed in second HPLC run (red (lower) chromatogram). The chromatograms of the two runs were then overlayed.